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The effect of plant species richness on biomass, carbon sequestration and potential for climate change mitigation on boreal zone grass cultivation in Finland

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Master's thesis

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Tiivistelmä – Referat – Abstract

Improving land management to mitigate climate change is important, especially in agriculture on soils with high organic content. Many studies have found evidence that increasing diversity can help to improve plant biomass production and soil carbon storage. This is attributed to complementarity which consists of more efficient resource use due to niche differences and facilitative interactions. For the total climate impact, the effect of greenhouse gas emissions from the soil needs to be considered.

To find out if adding more species to a grass mixture could have similar benefits in boreal zone grass cultivation in Finland, an experiment was set up with four different species mixtures, and three levels of species richness were established under a nurse crop. It was additionally of interest if these effects can counter the emissions of cultivation on organic soils. Biomass samples were collected both before the nurse crop was removed and at the end of the growing season. Both species richness and Shannon diversity index were considered as explanatory factors. Carbon exchange, divided into respiration and photosynthetic capacity, as well as nitrous oxide and methane fluxes, were monitored monthly.

There was no strong evidence that species richness affects biomass or greenhouse gas fluxes during the first year. The effect of species richness on the biomass was clearer when the diversity index was considered. These results were significant when the lowest biomass values were excluded from the analysis, probably because complementary resource use needs enough biomass to have an effect. The differences in carbon flux measurements may be sensitive to timing within the growing season since the results closest to significant were obtained at the start of the season. At the time, the measurement conditions were good and the nurse crop biomass was small enough not to obscure the effects of grass mixture. When it comes to other greenhouse gases, species richness had most impact on early nitrous oxide emissions, while methane flux probably needs significantly more time for any changes to appear. Overall, the effect of species richness needs to be studied over the full grass cultivation cycle to find out the full effect. Based on current results, increasing species richness may be an option when other methods cannot be used to reduce emissions and improve carbon sink of agriculture.

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Biomass, carbon sequestration, greenhouse gases, species richness, grassland cultivation, climate change mitigation, North Bothnia

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Kasvien lajimäärän vaikutus biomassaan, hiilensidontaan ja sen mahdollisuudet ilmastomuutoksen hillintään Suomen keskiboreaalisen vyöhykkeen nurmenviljelyssä

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Tiivistelmä – Referat – Abstract

Maankäytön käytäntöjen muuttaminen ilmastonmuutoksen hillitsemiseksi on tärkeää, etenkin maanviljelyssä maaperällä, jossa on runsaasti orgaanista ainetta. Useissa tutkimuksissa monimuotoisuuden lisääminen on parantanut biomassan tuotosta ja hiilivarastoa. Tähän on syynä ekologisten lokeroiden komplementaarisuus, eli erot, kuten kasvun ajoittumisessa ja ravinteiden tarpeessa, sekä lajien välinen positiivinen vuorovaikutus. Maaperän kasvihuonepäästöt on myös otettava huomioon, jotta kokonaisvaikutus ilmaston kannalta voitaisiin arvioida.

Neljän eri lajiseoksen ja kolmen eri lajimäärän koeruudut perustettiin suojaviljan (ohra) alle Pohjois-Pohjanmaalle lajimäärän lisäämisen hyötyjen selvittämiseksi Suomen keskiboreaalisella vyöhykkeellä. Lisäksi kiinnostuksen kohteena oli, miten hyvin suurempi lajimäärän voisi kompensoida multa- ja turvemaiden viljelystä syntyviä päästöjä. Biomassanäytteet kerättiin sekä ennen ohran korjuuta että kasvukauden lopussa lokakuussa. Sekä lajimäärää että Shannonin diversiteetti-indeksiä, joka ottaa huomioon myös lajien tasaisuuden, käytettiin selittävänä muuttujana. Hiilitasetta jaettuna hengitykseen ja fotosynteesiin sekä ilokaasu- ja metaanipäästöjä mitattiin kuukausittain.

Lajimäärän vaikutuksesta biomassaan ja kasvihuonekaasujen vaihtoon ei saatu vahvoja todisteita nurmenkierron ensimmäisenä vuonna. Lajimäärän vaikutus biomassaan oli selkeämpi, kun diversiteetti-indeksiä käytettiin selittävänä tekijänä, ja tulokset olivat tilastollisesti merkittäviä, kun pienimmät biomassamäärät jätettiin pois analyysistä. Syynä olivat luultavasti ulkoiset tekijät, jotka vaikuttivat kasvupaikalla enemmän kuin resurssien rajallisuus. Hiilivuomittausten ajankohdan merkitys saattoi olla huomattava, sillä lähimpänä tilastollisesti merkittäviä tuloksia päästiin kasvukauden alussa, kun olosuhteet olivat ihanteelliset ja ohran määrä ei vielä peittänyt nurmilajien vaikutusta. Muista kasvihuonekaasuista lajimäärä vaikutti hieman ilokaasupäästöihin heinäkuun alussa, kun taas mahdolliset vaikutukset metaanivuohon ilmaantunevat vasta huomattavan ajan kuluttua. Lajimäärän kokonaisvaikutuksen selvittämiseksi tutkimuksen pitäisikin kestää vähintään nurmen kiertoajan. Näiden tulosten perusteella lajimäärän lisäys saattaa toimia ilmastotoimena, kun muut vaihtoehdot eivät ole käytännöllisiä.

Avainsanat – Nyckelord – Keywords

Biomassa, hiilensidonta, kasvihuonekaasut, lajimäärä, nurmenviljely, ilmastomuutoksen hillintä, Pohjois-Pohjanmaa

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1. Introduction

1.1 Climate change mitigation

Climate change is a problem caused by anthropogenic carbon dioxide (CO_2) emissions and, to a smaller extent, land use changes. The land use changes, especially deforestation and wetland drainage, have released carbon from the soil and biomass and, reduced the capacity of ecosystems to sequestrate carbon. Between 1959 and 2020 19 % of the total CO_2 emissions were caused by land use changes (Friedlingstein et al. 2020). In addition to reducing emissions, removing carbon (C) from the atmosphere is important in order to achieve the goal of mitigate global warming through improved land management (Griscom et al. 2017; Seddon et al. 2020).

The mitigation potential of improving land management is based on using the natural C-cycle (Figure 1A) to remove C from the atmosphere, which is in essence the same process as the land use change induced warming but in reverse. Terrestrial plants take in CO_2 for photosynthesis and about 60 % thereof returns into the atmosphere through autotrophic respiration (Bonan 2008). The rest of the C is used to build up tissues, some of which end up in the soil as litter. Respiration and decomposition by the soil biota return the C back to the atmosphere at a rate which depends on the form of C and on the soil conditions (Bonan 2008).

In addition to C, soils emit two other greenhouse gases methane (CH_4) and nitrous oxide (N_2O). CH_4 is produced in anaerobic conditions by methanogens and used mostly in aerobic conditions by methanotrophs. When the methanotrophs are more active than the methanogens, the soil can act as a sink for CO_4. Nitrogen (N) cycle (Figure 1B) happens between the plant biomass and the soil, when N is getting mineralized from organic from in the litter to mineral N which is used by plants. N_2O is produced in nitrification and denitrification processes in the soil (Bonan 2008). The N_2O emissions from the soil have increased due to fertilisation, which leads to excess N when plants are not consuming less Paustian et al. 2016).

Soils are the largest terrestrial carbon pool and thus improving soil carbon pools have great potential for mitigating climate change (Paustian et al. 2016). In general, improvements in land management, along with conservation and restoration, have significant potential to climate change mitigation (Griscom et al. 2017). According to that analysis, the role of grasslands and agricultural lands is

significant, and rises to be the fourth most important one when considering cost-effective means of climate mitigation (Griscom et al. 2017).

One of the measures suggested is cultivating cover crops along the main crop. A significant benefit of cover crops is that the biomass is photosynthesising when the actual crop is not (Poeplau and Don 2015), and thus the period with bare soil remains short. Furthermore, cover crops reduce erosion and nutrient runoff during the less productive season (Poeplau and Don 2015). For example, in Nordic countries fallow agricultural peatlands produce more emissions in CO_2 equivalent than other ways of peatland management (Maljanen et al. 2010) so reducing the fallow period with cover crops could potentially be important in reducing emissions.

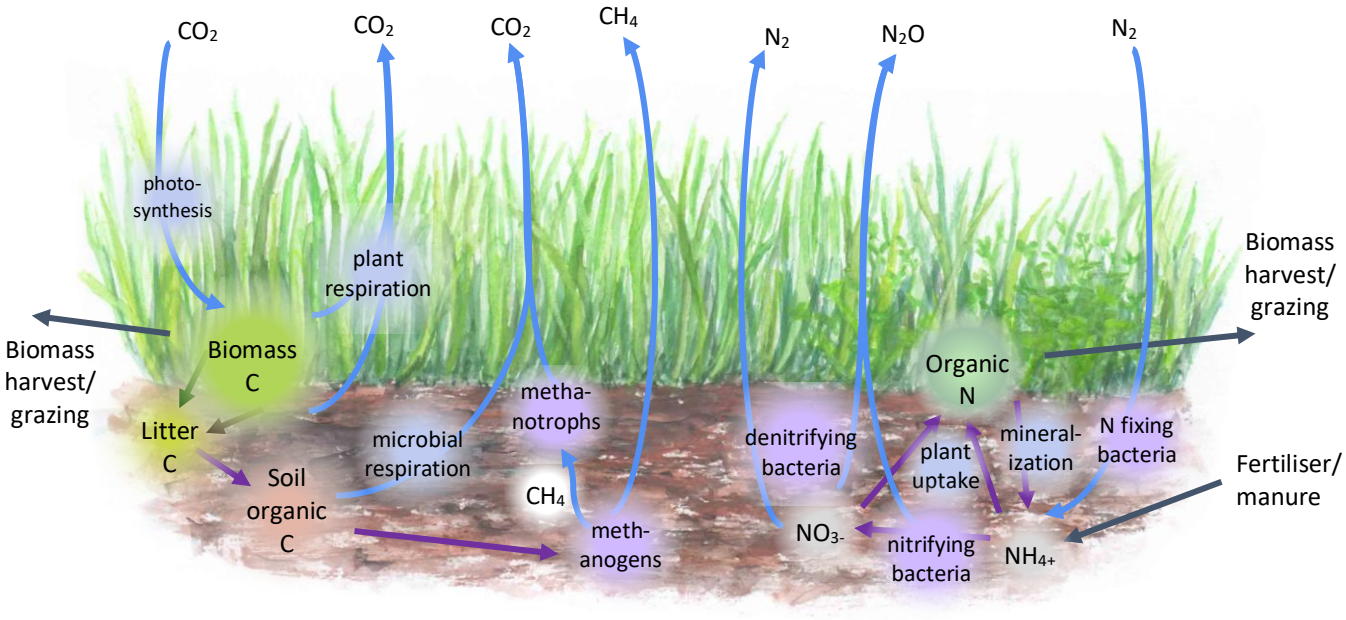

Figure 1. Carbon (A) and nitrogen (B) cycles

1.2 Cultivation on organic soils in Finland

In Finland, 85 000 ha, which corresponds to 0.8% of the total peatland area, was in agricultural use in 2000 (Turunen 2008). According to Maljanen et al. (2010), the agriculture on peat soils has a clear warming effect while agriculture on mineral soil could have a cooling effect. However, the loss of

soil C has been observed also in agriculture on mineral soils in Finland (Heikkinen et al. 2013). There has been some shift in cultivation towards organic soils since 1990 (Kekkonen et al. 2019) which can be regarded as unsatisfactory development from the climate perspective.

The greenhouse gas emissions are higher on agricultural peat soils than in mineral soils because the peat decomposition in drained sites leads to loss of CO_2, along with nitrous oxide emissions due to the high N content in peat (Maljanen et al. 2010). Because of this, the reduction of cultivated peat soils can be seen as efficient targets for climate warming mitigation in Finland (Kekkonen et al. 2019).

Most of the carbon lost from peatlands through gas emissions comes from agricultural soils (Turunen 2008). Of the total agricultural land in Finland, the proportion of mould and peat soils is 9.7 % and 3.8 % respectively (Myllys and Sinkkonen 2004). However, 43.6 % of all the N_2O emissions from agricultural soils originated from cultivation of organic soils in 2017 (Forsell et al. 2019). Peat fields in Finland are the most abundant in Lapland and Bothnia, and mould fields converted from peat are the most abundant in Bothnia (Myllys and Sinkkonen 2004) where grass cultivation is the dominant form of agriculture.

The majority of organic field area is used for cultivating grass for forage (Lehtonen et al. 2021). This is better for the climate than cultivating annual crops but there is still potential to reduce emissions (Lehtonen et al. 2021). Remote and nutrient-poor organic fields are relatively easy targets for the climate mitigation (Lehtonen et al. 2021). However, removing all peatland fields from cultivation to reduce the emissions would result in a great regional effect, making it politically difficult. For climate change mitigation it is more effective to afforest organic peat land or increase the level of groundwater for continued cultivation or restoration of wetland (Kekkonen et al. 2019, Lehtonen et al. 2021). Thus, it is necessary to find ways to reduce the emission while keeping these fields in cultivation. In addition, the older cultivated fields have already lost a significant part of their organic material, so restoration is not as useful as adapted cultivation (Kekkonen et al. 2019). In this study, the potential of increasing the number of species cultivated is considered from this point of view.

1.3 Plant diversity effect on carbon sequestration and yield

Biodiversity is divided into diversity of ecosystems, species within an ecosystem and genetic diversity. Here the focus is mainly on species diversity within an ecosystem, which is usually considered to mean the combination of species richness, i.e. the number of species present, and evenness of their distribution (Peet 1974). Species richness alone is not necessarily a good measurement, since it does not show how the biomass is divided between the species, which can be more important for the function of the ecosystem (Keddy 2017). The combination of these two can be measured using diversity indexes, which have different weightings on species richness and abundance (Morris et al. 2014). Additionally, functional diversity can be used, classifying the species into functional groups based on, for example, morphology, physiology or symbiotic association, depending what is relevant in the ecosystem in question (Körner 1994). This diversity can be divided into similar components as species diversity (Norman et al. 2005).

The theoretical benefit of diversity is a complementary effect (Hooper 1998, Loreau and Hector 2001). This can be facilitation between species or complementary use of resources due to niche differentiation (Loreau and Hector 2001), which allows the species present to more fully use the resources present in the ecosystem. For example, legumes can facilitate growth of other species as a result of symbiosis with nitrogen-fixing bacteria leading to nitrogen-rich litter being added to the soil. Complementary use of resources in this case means that, because of N fixation, legumes do not compete of soil N as much. Cooccurring species may be strong competitors as long as they have differences in timing of growth, photosynthesis, and rooting depth (Grime 1998).

Biodiversity has been suggested to have several benefits in a range of ecosystems (e.g. Isbell et al. 2017) which are discussed in more detail later in this chapter. Taking diversity into account in climate mitigation is necessary to ensure long term benefits of ecosystem restoration (Seddon et al. 2020). Maestre et al. (2012) found that globally, plant species richness on drylands is a good predictor of ecosystem functions, especially those related to C sequestration and maintaining soil fertility.

In the context of climate change mitigation, certain diversity benefits are especially important. In a meta-analysis of forests, the productivity of trees was greater in mixtures than in monocultures with a more pronounced effect in high-precipitation areas (Jactel et al. 2018). Species richness increases grassland yield at least in less intensively fertilized ecosystems (Isbell et al. 2013; Isbell et al. 2017).

In two species richness experiments in grassland ecosystems in Germany and Minnesota (USA), the forage yield in both cases had the clearest response to species richness (Isbell et al. 2017). The higher number of species leads to a higher above-ground biomass (Fornara and Tilman 2008). Ecosystem productivity in hay fields sown with higher species richness is shown to remain on the higher level for over eight years (Bullock et al. 2007). The increased productivity could allow some reforestation or reduce the need of expansion of agricultural lands.

The interactions between diversity and nutrient availability are important to consider. In highly managed ecosystems, competitive exclusion has been suggested to lead to a lower species richness (Grime 1973). Isbell et al. (2013) found that the addition of nitrogen caused plant diversity to diminish over time, and this effect countered the initial improvement in plant productivity. The mechanism for improved productivity is different species having different resource requirements (Hooper 1998, Catovsky et al. 2002). There is a possibility that adding diversity could reduce both the need for fertilization and the emissions related to nitrogen. The effect of fertilisation is different in created and natural nutrient gradients (Rajaniemi 2003), so the effect on an artificially created community can also differ.

Plant diversity can also improve carbon sinks by adding more carbon in the soil. Restoring grassland plant diversity has been found to improve soil carbon storage in Minnesota, the effect getting clearer over time (Yang et al. 2019). Species mixtures have been found to accumulate about five times more carbon to the topsoil than the monocultures of the species in that mixture (Fornara and Tilman 2008). This is because diversity, especially that of functional groups, increases root biomass (Fornara and Tilman 2008). However, according to De Deyn et al. (2011), the benefits are not necessarily a result of the number of species, but the addition of single species can be more significant.

Nevertheless, there is a need to examine if the net effect of diversity promotes climate change mitigation in all ecosystems. In theory, increase in plant diversity could accelerate the decomposition processes by leading to more and more stable soil substrate, as well as, increased diversity and biomass of microbial community (Catovsky et al. 2002). Since the effect of higher diversity on soil microbiota is complicated (Hooper et al. 2000), it could also lead to increased emissions of other greenhouse gases. For example, in a laboratory-scale experiment of constructed wetland, species richness increased methane emissions (Zhang et al. 2012). N_2O emissions could be

reduced with more organic matter in the soil and with better nutrient capture, while increased emissions due to higher N turnover rate are also possible (Blesh 2019). However, rather than species richness, N_2O emission might be more dependent on presence of certain species which have high productivity or the root length to capture nitrogen when it is abundant (Abalos et al. 2014). On the other hand, a combination of less productive species could slow down soil N cycle and, reducing N_2O emission, when mineral N is not as available (Abalos et al. 2014).

In many ecosystems, increasing the number of plant species leads to more bacterial biomass and soil respiration, this effect being more pronounced in the colder climates (Chen et al. 2019). However, in the grassland experiment in Germany, the microbial growth and turnover increased more than the respiration when plant species richness was higher, explaining a part of soil organic matter increase through an increase in dead microbial biomass (Prommer et al. 2020). The diversity aboveground does not necessarily always increase the diversity of soil organisms (Hooper et al. 2000). Even though there is ample evidence that species richness can make the plant communities more efficient carbon sinks, the total effect on climate needs to be verified in different soil and climate conditions.

In addition, species diversity reduces vulnerability to extreme conditions and improves the recovery of plant communities on grasslands (Tilman and Downing 1994). In agriculture, diversification reduces herbivore damage by making feeding the areas less continuous and increasing the abundance of species controlling herbivore populations (Letourneau et al. 2011). However, the yields were lower in a diverse cultivation than in a monoculture due to part of the main crop being replaced to make space for diversification (Letourneau et al. 2011). Since the new species added to grass fields contribute to the yield, increasing the number of species is easier to justify.

1.4 Aims

This thesis aims to find out whether carbon sequestration and biomass increase with species richness in northern grass cultivation in Finland. Typically in this area the grass is established under a nurse crop such as barley or oat (Hakkola 1978) to protect the grass from heat and to reduce the growth of weeds, while growing fast to give harvest already during the first year. Here the focus is on the establishing year of the grass and whether the positive effects of species richness are already

present on a peat-derived land. In addition, other greenhouse gases from the soil are considered, to find out if these effects could decrease the warming effect of fields on organic soils. For this end, gas exchange between the field and atmosphere was measured with chamber method monthly for four different species mixtures. Additionally, aboveground plant biomass samples were measured by species on two occasions. In these mixtures, the shares of functional groups are approximately even, so the potential effect would be primarily that of species richness.

The first hypothesis is that (1) the higher number of species results in higher aboveground plant biomass. There is a possibility that adding more productive species to a mixture could explain the increased biomass. To account for that, the mixture with the highest number of species is a combination of the other mixtures, so the number of species is more important than which species are selected. In addition, the share of species is considered since if only a few individuals of a species are present, the effect they have is likely diminished.

The second hypothesis is that (2) the possible changes is biomass are reflected in the carbon fluxes between the field and the atmosphere. The measured net carbon fluxes are partitioned into net ecosystem respiration and gross photosynthesis or gross primary production (GPP). This is done because soil heterogeneity can lead to different levels of respiration despite the species present. Other greenhouse gases (CH_4, N_2O) are considered to see if they have can counter the potential climate benefits rather than calculating the total effect on climate. The third hypothesis is that (3) the number of species present affect the fluxes of other greenhouse gases. Additional soil and weather data are used as background information for these measurements.

2. Materials and methods

2.1 Study setup

The study site is in Ruukki, Siikajoki (64° 41' 1" N, 25° 6' 42" E) on a drained peatland with about 45 cm layer of mainly organic material. The original peat has decomposed during the approximately 100 years of cultivation and the soil is now classified as mould (Honkakoski M, personal communication 2020). The yearly mean temperature between 1989–2018 was 3.2 °C and total annual precipitation 548 mm (FMI data). The soil contains 623 t C ha^{-1} and 36.4 t N ha^{-1} (Yli-Halla M,

personal communication 2020). The weather data from 1989 to 2019, provided by the Finnish Meteorological Institute (FMI), was available from a weather station about 500 m away from the site.

The experimental setting consists of 4 treatments: 2 species, 5 species, different 5 species, and combined 10 species (Table 1). There were four replicates, to which each treatment was randomly assigned. The species in the first treatment were *Phleum pratense* and *Trifolium hybridum*; in the second *Phleum pratense*, *Schedonorus arundinaceus, Festuca rubra*, *Trifolium repens*, and *Medicago sativa*; in the third *Schedonorus pratensis, Phalaroides arundinacea*, *Poa pratensis*, *Trifolium hybridum*, and *Cichorium intybus*. The species mixtures were established as cover crop for spring barley *Hordeum vulgare* 'Brage'. 20 % share of the seed mass in each mixture was allocated to legumes.

The sowing took place on June 13th 2019 in plots of 8 x 1,5 meters (Figure 2). Plots 1–8 and 9–16 were side by side with an eight-meter corridor between them. The seeds were sown with a fertilizer

Table 1. Species in the mixtures and the share of seed mass (%)

mixture 1	mixture 2	mixture 3	mixture 4
Phleum pratense (80%)	*Phleum pratense* (30%)	*Schedonorus pratensis* (30%)	*Phleum pratense* (15%)
Trifolium hybridum (20%)	*Schedonorus arundinaceus* (30%)	*Phalaroides arundinacea* (20%)	*Schedonorus pratensis* (15%)
	Festuca rubra (20%)	*Poa pratensis* (20%)	*Schedonorus arundinaceus* (15%)
	Trifolium repens (10%)	*Trifolium hybridum* (20%)	*Phalaroides arundinacea* (10%)
	Medicago sativa (10%)	*Cichorium intybus* (10%)	*Poa pratensis* (10%)
			Festuca rubra (10%)
			Trifolium hybridum (10%)
			Trifolium repens (5%)
			Medicago sativa (5%)
			Cichorium intybus (5%)

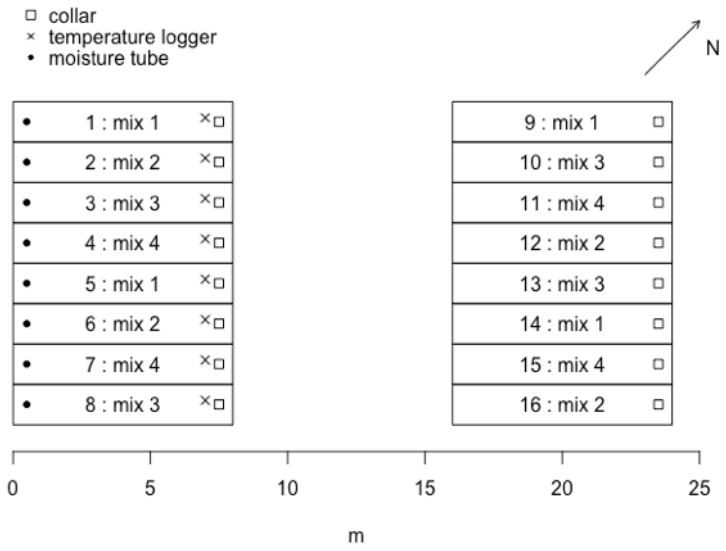

Figure 2. The experimental plots with the location of the collars, temperature loggers and moisture tubes. The different mixtures (mix) are explained in Table 1

(402 kg ha^{-1}, YaraMila Y4), at a depth of 5–6 cm and 2–4 cm for barley and for the rest of the species, respectively, and the amount of seeds sown was 141 kg ha^{-1} for barley and 35 kg ha^{-1} for the grass mixtures (Honkakoski M, personal communication 2021). The surrounding field was treated on July 25th with a gibberellic acid synthesis inhibitor to limit the height of the barley, but the study site was left untreated. On August 30th, the barley was harvested.

2.2 Field measurements

The chamber measurements were conducted manually on permanently installed steel collars (0.545 m x 0. 545 m inside the collar) set on the north-east end of each plot. The height of each collar was measured from ten points with a tape measure in order to calculate the volume of air inside on June 17th, September 10th and October 10th. The measurements for the light response of net CO_2 exchange were conducted once a month from June to October with a 59 cm x 59 cm x 80 cm transparent chamber (Figure 3). The chamber had an L-shaped lid which covered the top and one wall of the chamber, to enable more efficient flushing between the measurements. There were two fans inside the chamber to make sure that the air was well mixed.

During a single closure and in ten-second intervals, photosynthetically active radiation was measured with one sensor (PAR) (PQS1, Kipp & Zonen, The Netherlands), and air temperature, pressure and relative humidity inside the chamber were measured with another sensor (BME280, Bosch Sensortec, Germany). CO_2 concentration inside the chamber was measured using a near-infrared gas analyser (Li840a, LI-COR, USA), set to measure every five seconds. The aim was to

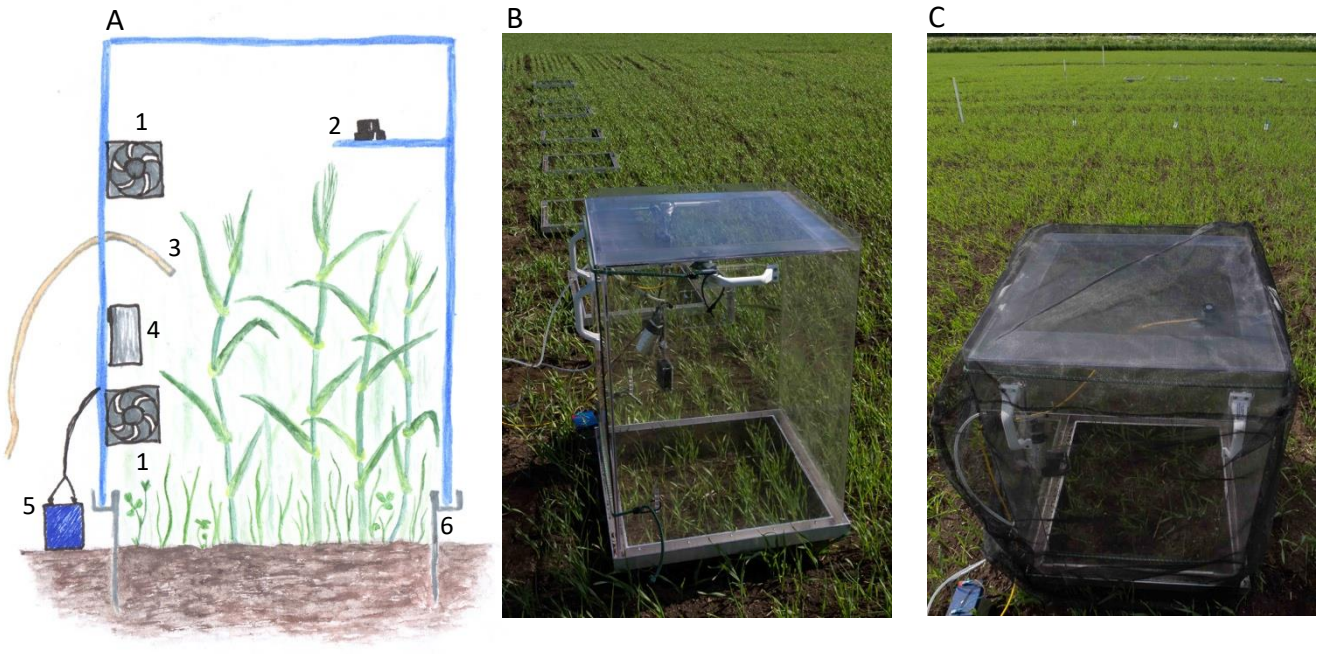

Figure 3. (A) Transparent chamber setup: 1. fans, 2. PAR-sensor, 3. air tube to the gas analyser, 4. temperature, moisture, and pressure sensor, 5. battery for the fans, 6. the collar. (B) Unshaded chamber, (C) shaded chamber on June 28th 2019

repeat the measurement in five different light intensities that would stay stable enough during each chamber closure of two to three minutes. The chamber was shaded to get the lower intensities by covering it with one to three layers of transparent fabric or, for respiration, a non-transparent one. Between the closures, the chamber was aired.

Methane and nitrous oxide exchange were measured from the same collars with a 60 x 60 x 76 cm opaque chamber connected to a gas analyser (DX4015 FTIR Gas Analyzer, Gasmet, Finland) for eight minutes (Figure 4). There were two fans inside the chamber and the chamber was aired before moving to the next collar. In addition, there was a temperature logger (HOBO 64K Pendant Temperature Data Logger, Onset, USA) inside the chamber. The pressure reading closest to the middle of the closure from the weather station was used (Vekuri H, personal communication 2020).

The soil temperature was measured at 5, 10 and 20 cm depths beside each collar along the chamber measurements. Additionally, temperature loggers (HOBO 64K Pendant Temperature Data Logger, Onset, USA) were buried on half of the plots on July 3rd, and the data was extracted on spring 2020. Soil moisture content was measured (PR2/4 Profile Probe, Delta-T Devices, UK) weekly, at 10, 20, 30 and 40 cm depths, from July 7th until the end of August and after that once in September and October. Because of stones or dense mineral soil layer, some of the tubes were higher up, so the

Figure 4. Greenhouse gas measurement chamber on June 17th 2019. Black tube takes the air from the chamber to the gas analyser in the bottom left corner, and the white tube returns the air back to the chamber.

actual measurement depths were defined by measuring the part of the tube above ground with a tape measure and the moisture readings were reclassified to 10 cm groups. The growth of barley was monitored by measuring the length of one representative individual with a tape measure in each collar weekly between June 25th and August 16th.

The first aboveground biomass samples were taken inside a 25 x 25 cm frame on August 12th from each of the plots. Second samples were taken during October 12th and 13th with a 25 x 40 cm frame. The placement of the frame was systematic to have similar edge effect on all of the samples. Barley was cut 1 cm above ground level and the other species from ground level. To get the shares of species in the mixtures and the combined share of other species present, the samples were sorted, dried first to air dryness and later for one hour in 60 °C, before weighing to get dry biomass per ground area.

2.3 Gas exchange and light response calculation

The gas exchange rate for each closure was defined based on the gas concentration change inside the chamber. Gas exchange rate F (µg m^{-2} s^{-1}), was calculated for each closure (Equation 1).

Equation 1 $$F = \frac{dC}{dt} M P V \frac{1}{R T A}$$

In Equation 1, dC/dt is the rate of gas concentration change over time (ppm s^{-1}), V is the combined volume of the chamber and the collar (m^3), and the volume of the collar was linearly interpolated

between the times its height was measured; P is the ambient atmospheric pressure (Pa) and T is the temperature (K) inside the chamber, A is the collar area (m^2), and R is the gas constant, 8.314 m^3 Pa K^{-1} mol^{-1}. M is the molar mass, 44.01 g mol^{-1} for CO_2, 16.04 g mol^{-1} for CH_4, and 44.01 g mol^{-1} for N_2O.

For some of the light chamber results, a more stable period within the closure was used instead, the minimum time acceptable for a closure being 50 seconds. A closure was discarded if the standard deviation of PAR was higher than 150 µmol m^{-2} s^{-1}, allowing only short or minor changes in illumination due to clouds. The linear slope of change in CO_2 over time was fitted to a single closure and outliers removed with Random Sample Consensus algorithm (Fischler and Bolles 1981; Pedregosa et al. 2011). However, if the difference between the minimum and maximum CO_2 concentrations was under 4 ppm, the variation in CO_2 level was assumed to be noise and the slope was calculated based on all of the observed concentrations. In addition, slopes with normalized root mean square error over 0.06 were discarded.

For the light chamber results, the light response curve of net ecosystem exchange of CO_2 (NEE) was fitted with the method of least squares (Figure 5). For the fitting, at least 4 gas exchange rates and the corresponding PAR were used to get estimates and standard errors for parameters of the curve (Equation 2, Equation 4).

Equation 2 $$\mathrm{NEE(PAR)} = \mathrm{P(PAR)} - \mathrm{R_{eco}}$$

In Equation 3, NEE (g CO_2 m^{-2} s^{-1}) is net ecosystem exchange of carbon, R_{eco} (g CO_2 m^{-2} s^{-1}) is ecosystem respiration, and P(PAR) is the gross primary production – i.e. photosynthesis – at a given PAR level (g CO_2 m^{-2} s^{-1}). The biological sign convention is used for NEE, meaning that the positive values indicate carbon gained to the ecosystem, while negative values show carbon lost into the atmosphere. The light response curve of photosynthesis is described as a saturating function of PAR, as follows:

Equation 4 $$\mathrm{P(PAR)} = \frac{\alpha\ \mathrm{GP_{max}}\ \mathrm{PAR}}{\alpha\ \mathrm{PAR} + \mathrm{GP_{max}}}$$

In Equation 3, GP_{max} (g CO_2 m^{-2} s^{-1}) is the gross primary production when PAR (µmol m^{-2} s^{-1}) approaches infinity. Parameter α is a coefficient that defines the curvature. Based on the equations

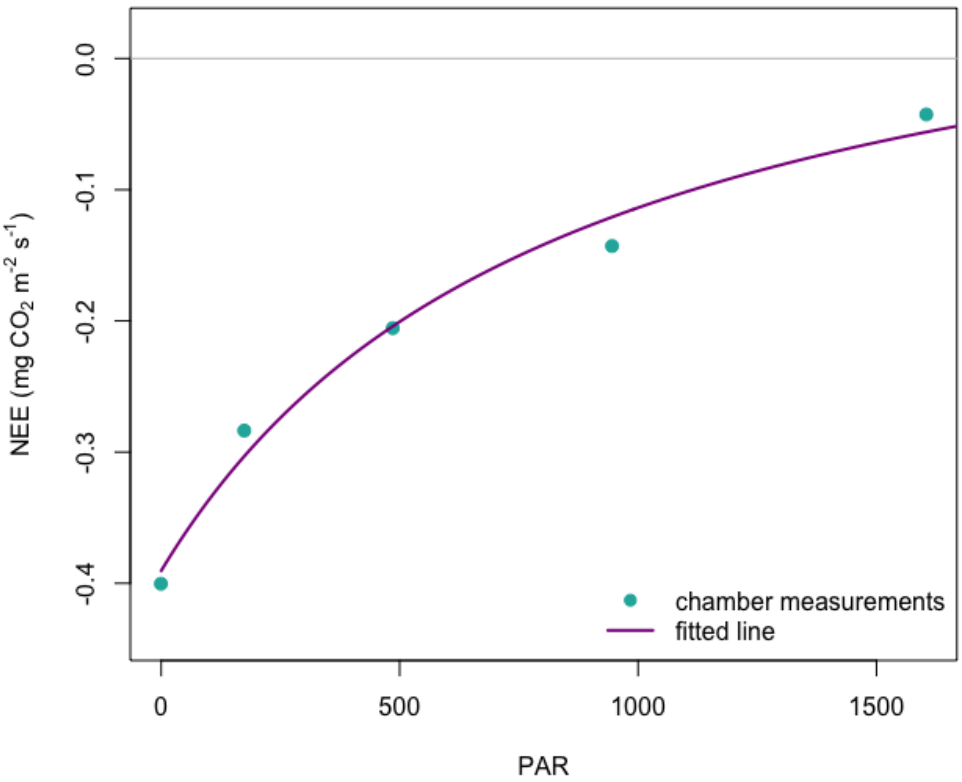

Figure 5. An example of light response of net ecosystem exchange (NEE) of CO_2 in collar 2 on June 27th 2019. Dots are measurements in the different light intensities obtained using the different shades and the line is the fitted curve (Equation 2, Equation 4).

2 and 3, the net exchange for a given PAR level (NEE(PAR)) can be calculated. For further analysis, the rate of photosynthesis for each plot and repetition was calculated using PAR values of 1000, 500 and 250 µmol m^{-2} s^{-1}.

2.4 Statistical analysis

The analysis is done with an analysis of variance (ANOVA) method on parameters of linear model, with the natural logarithm of the number of species in the treatment as an explanatory variable. Because the effect of adding species is likely to be greater in the beginning, the most likely relationship between number of plant species and ecosystem function is asymptotic (Vitousek and Hooper 1994). This means that after adding enough species, the benefit of complementary resource use diminishes since the limit of more efficient resource use is reached. For light response results, the respiration and the gross primary production at PAR level of 1000 µmol m^{-2} s^{-1} are examined separately. To reduce uncertainty for the months with less radiation results of PAR 500 µmol m^{-2}

s^{-1} are also considered in a separated analysis. The significance level used is 0.05. The months with less than 12 chamber measurement results were excluded from the analysis, leading to the light response results of October and the N_2O and CH_4 results of August being discarded.

Besides the number of species, the Shannon's diversity index (H', Equation 5) was used as an explanatory variable for the biomass.

Equation 5 $$H' = -\Sigma\, p_i \ln p_i$$

In Equation 4, p_i is the proportion of biomass of species i (Shannon 1948, see Morris et al. 2014). Because of relatively short time the cover crops had to grow after the cutting of the barley, the possibility of a threshold biomass, after which correlation emerges, is tested by excluding some of the smaller biomass samples. Setting the threshold to 60 g m^{-2} removes 5 samples from the data, the two lowest from mixture 1 and one lowest from the rest of the mixtures.

3. Results

3.1 Weather and soil conditions during measurements

The monthly average temperatures were mostly within the standard deviation of the past 30 years, the exceptions to this being before the start of the field measurements, i.e. January and April (Figure 6A). The monthly precipitation in July was lower than during the past three decades (Figure 6C), of only 2.3 mm (all during July 7th). This was followed by a 34-days rainless period that extended into August (Figure 6D). Despite this, the monthly precipitation in August was the fifth largest in the past 30 years, including the highest daily precipitation of the year on August 12th, 30.2 mm.

The dry period affected the soil moisture, which was first evident between 10 and 20 cm depths (Figure 7). Towards the end of July, a clear effect was also observable in the depth between 20 and 30 cm. In the 10–20 cm depth class, the moisture content fell below 20 per cent in all plots in the end of July and stayed about the same until the rain. In 20–30 cm depth, the moisture content degreased more gradually below 30 %. In 30-40 depth, the moisture content was generally above 40 % and the drought resulted in drop below this moisture content in some of the measurement points.

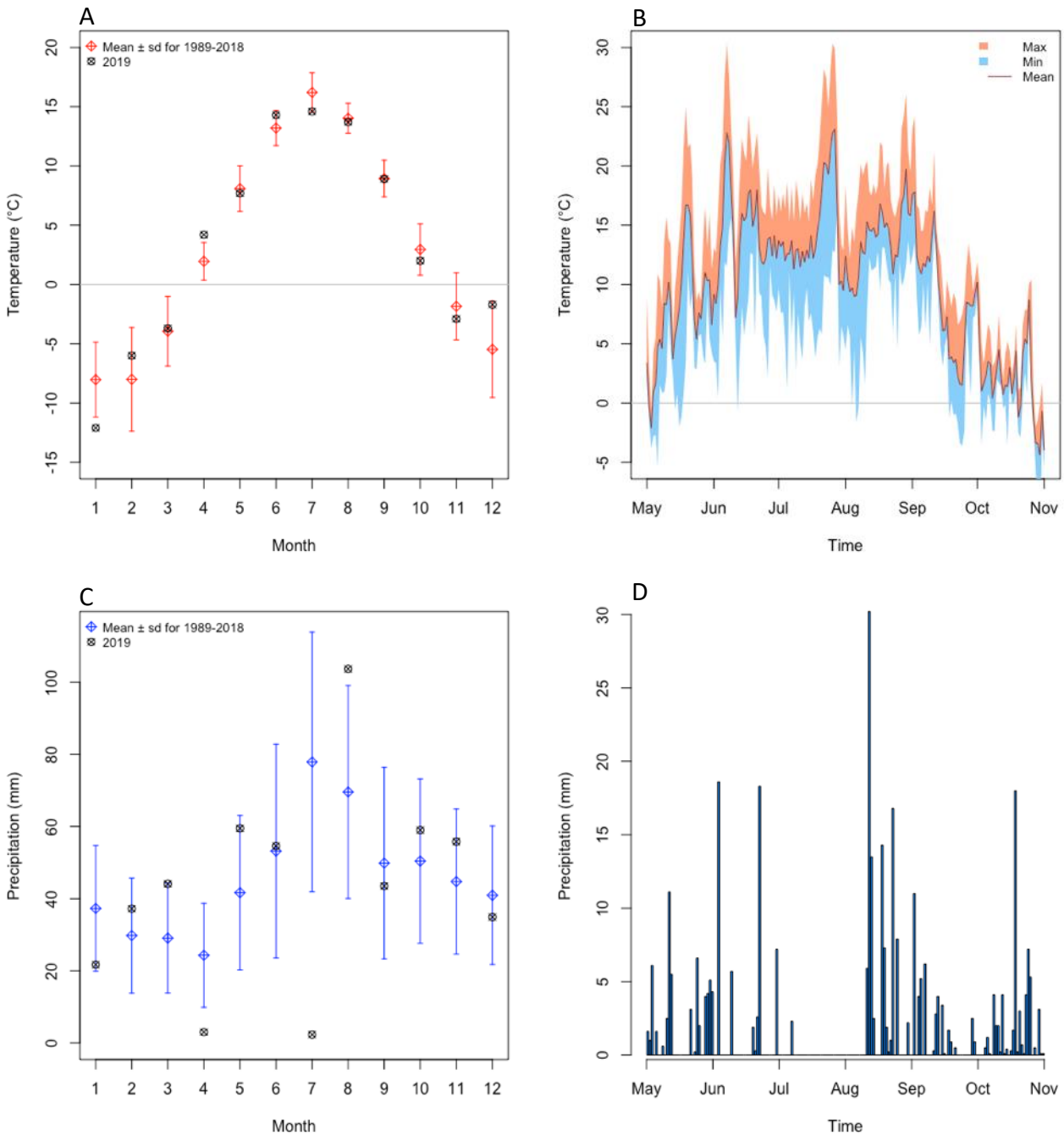

Figure 6. Conditions during measurements and comparison to previous years. (A) Monthly temperature on 2019 (black) and the previous 30 years (red) ± standart deviation. (B) Daily minimum, maximum, and mean temperatures from May to November 2019. (C) Monthly precipitation on 2019 (black) and the previous 30 years (blue) ± standart deviation. (D) Daily precipitation from May to November 2019. Data from the FMI meteorological station in Ruukki.

The soil temperature measured along the chamber measurements followed quite well the daily mean air temperature in all three depths (Figure 8). As to be expected, the deeper measurements were more stable and, in the presence of barley, even the variation in 5 cm depth temperatures was smoother due to shading.

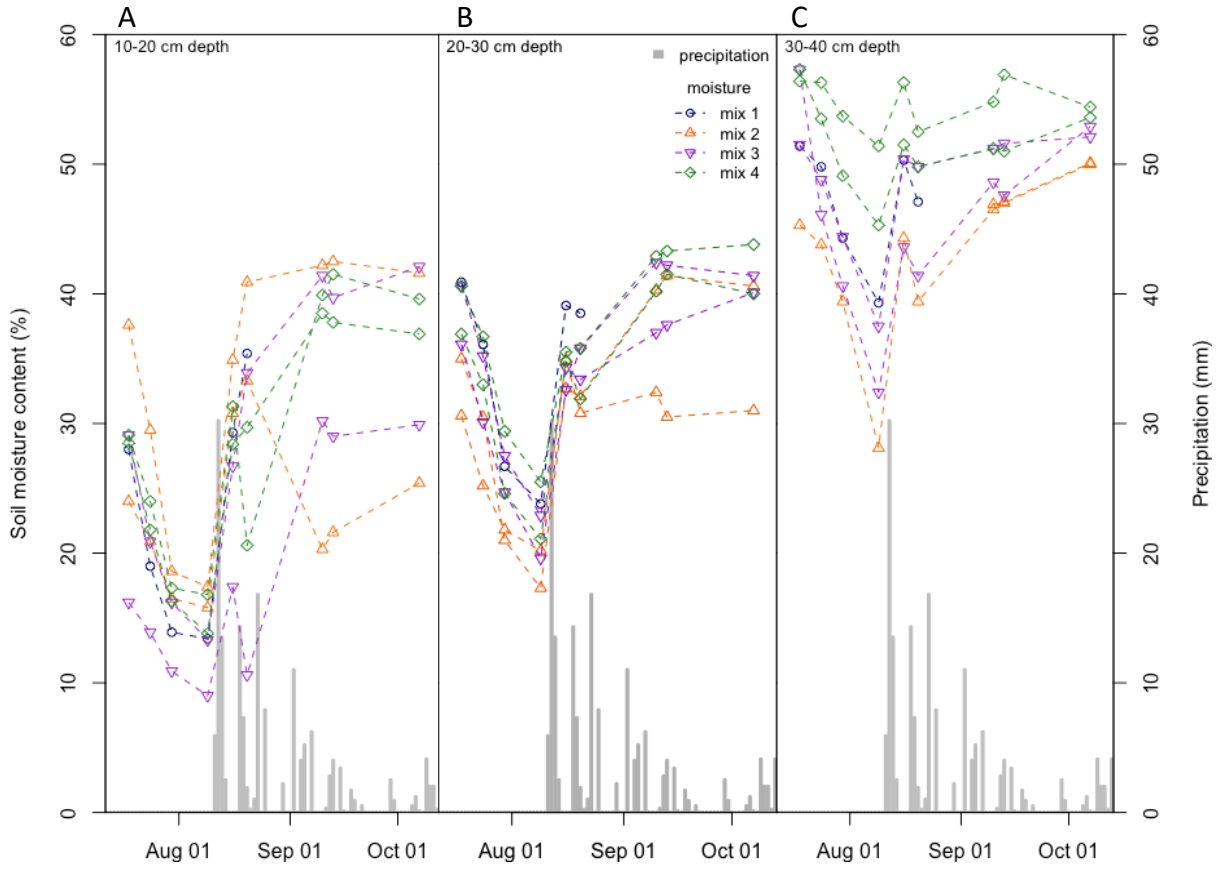

Figure 7. Daily precipitation and soil moisture in 4 different grass species mixtures in (A) 10–20 cm, (B) 20–30 cm and (C) 30–40 cm depths from July 15th to October 10th

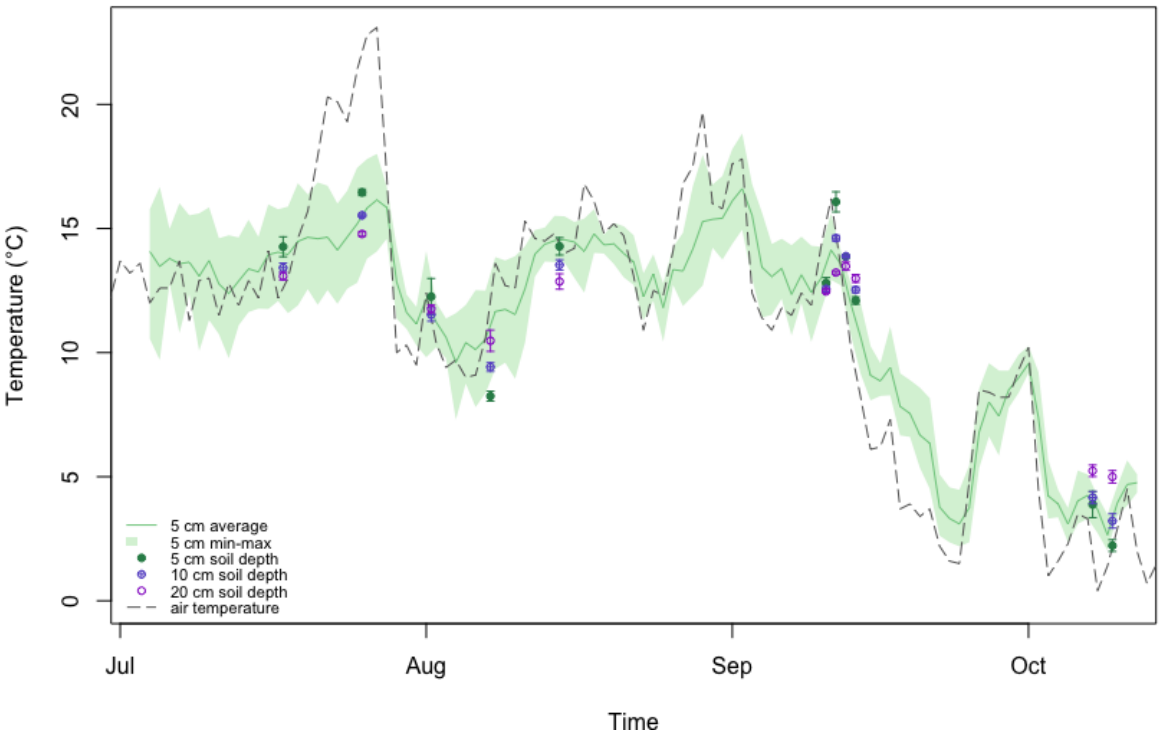

Figure 8. Air and soil temperature average (7 loggers) of daily mean and variation at 5 cm depth in line, and points indicating mean ± sd of measurements in 5, 10 and 20 cm depth, from July 15th to October 10th 2019.

3.2 Growth and biomass

The growth of barley was fastest towards the end of July (Figure 9), resulting in an average dry matter biomass of 674 g m^{-2} in August. The grass mixture did not have an observable effect on the growth of barley. The biomass of the cover species was small and their biomass varied between 4.00 and 48.0 g m^{-2} in August (Figure 10B). However, almost all the species were established enough to be present in all or almost all of the biomass samples with maximum one missing. The exception to this was Poa pratensis which was present in only one of the eight samples it was expected in. Unsown species present were *Elytrigia repens* (mean 9.85 g m^{-2}, sd 24.9 g m^{-2}) and *Alopecurus geniculatus* (mean 1.14 g m^{-2}, sd 2.65 g m^{-2}).

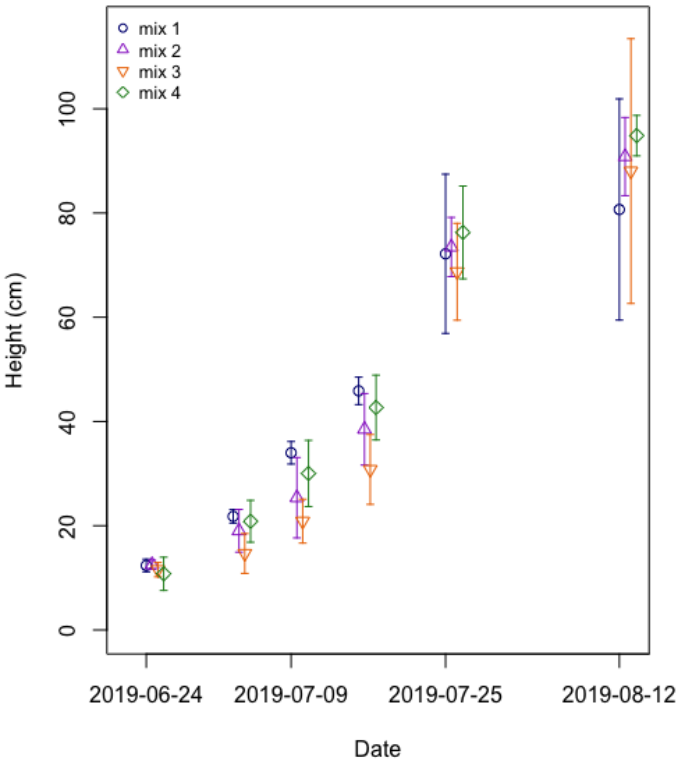

Figure 9. Height of barley, mean of each mixture ± standard deviation in all four mixtures from June 24th to August 12th.

In August, the biomass of barley in mixture 3 was slightly higher (734 g m^{-2} with standard deviation of 80.0 g m^{-2}) compared to mixture 4 (684 g m^{-2}, sd 85.4 g m^{-2}) (Figure 10A). A minimum of 83 % of biomass in every sample was barley. Neither the barley biomass nor the total biomass were significantly different between the treatments when explained with the logarithm of the number of species ($p > 0.1$).

In October, the highest average biomass was observed in the ten-species mixture and lowest in the two-species mixture (Figure 10C) but the difference was not statistically significant. *Poa pratensis* was present in all of the biomass samples it was expected in and, additionally, in some of the plots it was not sown into (Figure 10D). In addition to *E. repens*, and *A. geniculatus*, there was some barley sprouting again. However, the biomass did not statistically differ significantly between the treatments in the ANOVA ($p = 0.119$).

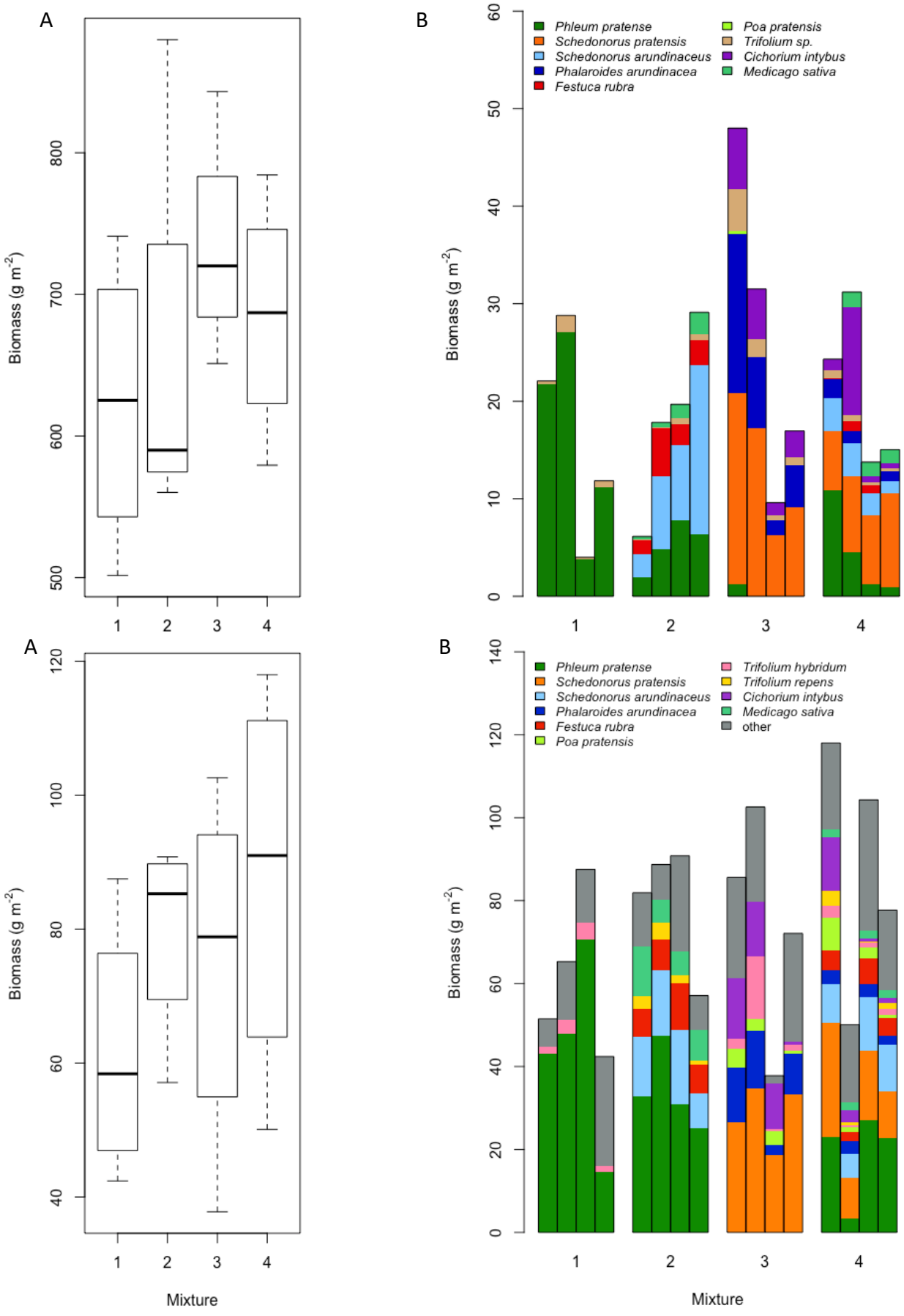

Figure 10. (A) Distribution of barley biomass and (B) biomass by species on August 12th. (C) Distribution of total biomass and (D) total biomass by species on October 13th.

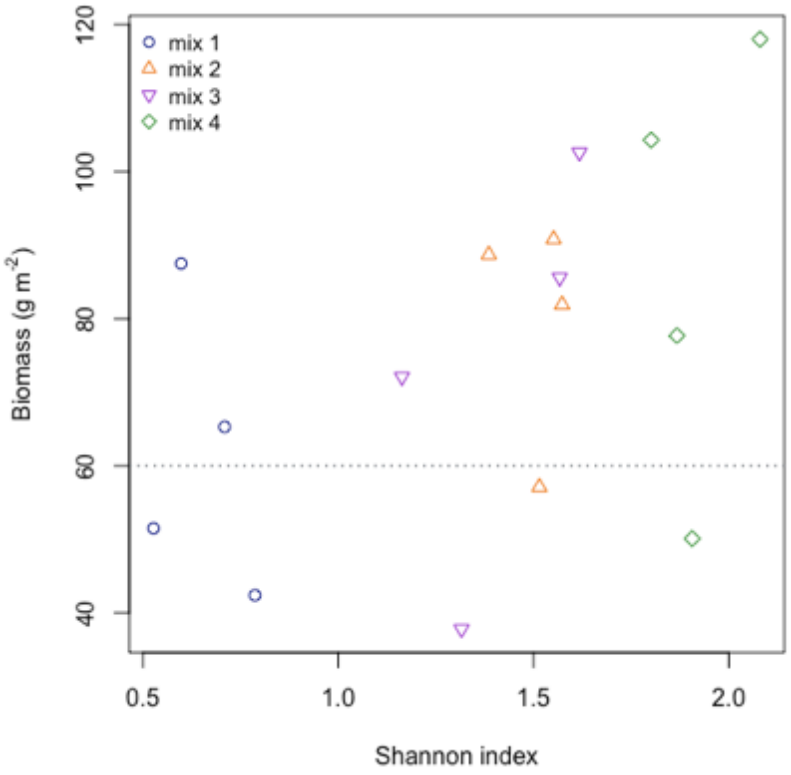

Figure 11. Total biomass in October against Shannon index in the different mixtures, with the dotted line indicating the 60 g m^{-2} threshold.

Using the Shannon index as the explanatory factor, the p-value was closest to significant, 0.0676 (Figure 11). However, there would be a significant relation between the Shannon index and the total biomass if the five samples, with poor growth and the total biomass under 60 g m^{-2}, were discarded from the analysis (p = 0.039).

3.3 Gross primary production and respiration

The photosynthetic capacity at different light levels was highest in July, and returned to the same level it started in June by October (Figure 12). Respiration followed similar pattern with lower levels in autumn (Figure 13). Overall, the number of species did not explain well the observed variation in NEE and GPP in ANOVA when PAR was 1000 µmol m^{-2} s^{-1} on each month. However, the GPP measurements of June came closest to significant with p = 0.0651, with highest GPP in mixture 3 (0.497 mg CO_2 m^{-2} s^{-1}) and lowest in mixture 1 (0.107 mg CO_2 m^{-2} s^{-1}). With a PAR level of 500 µmol m^{-2} s^{-1} in GPP of June, the difference is still the closest to significant, but not as close as for the PAR level of 1000 (p = 0.0969).

The differences in respiration between the treatments were not significant either. However, a slight pattern seemed to emerge, with the order from highest respiration to the lowest being the same in

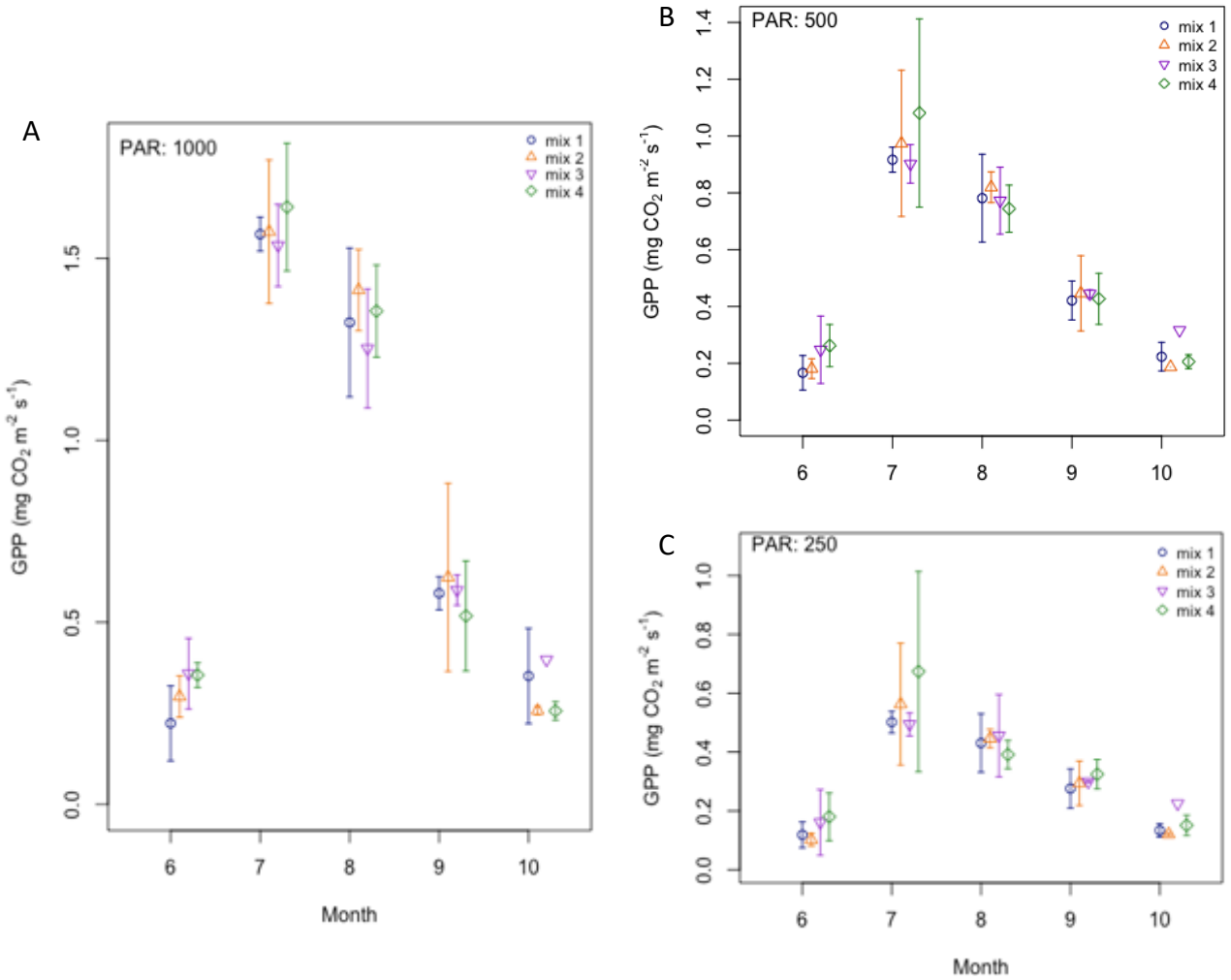

Figure 12. Gross primary production (GPP) mean ± standard deviation for all mixtures when photosynthetically active radiation is A) 1000, B) 500 and C) 250 µmol m-2 s-1

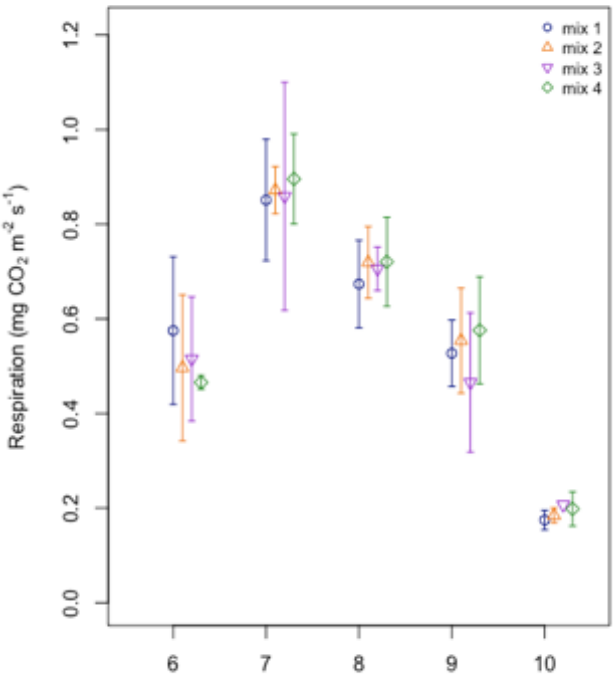

Figure 13. Ecosystem respiration mean ± standard deviation for all mixtures

July and August and almost the same in September. Overall, at light intensity of 1000 µmol m^{-2} s^{-1} the plots acted as net sources of CO_2 in July, a strong sink in July and August with the barley, and small sinks in September and October after the barley was removed (Figure 14). In September, these results are mostly extrapolated (max unshaded PAR 1170 µmol m^{-2} s^{-1}, mean 680 µmol m^{-2} s^{-1}), and in October theoretical (max unshaded PAR 473 µmol m^{-2} s^{-1}, mean 335 µmol m^{-2} s^{-1}).

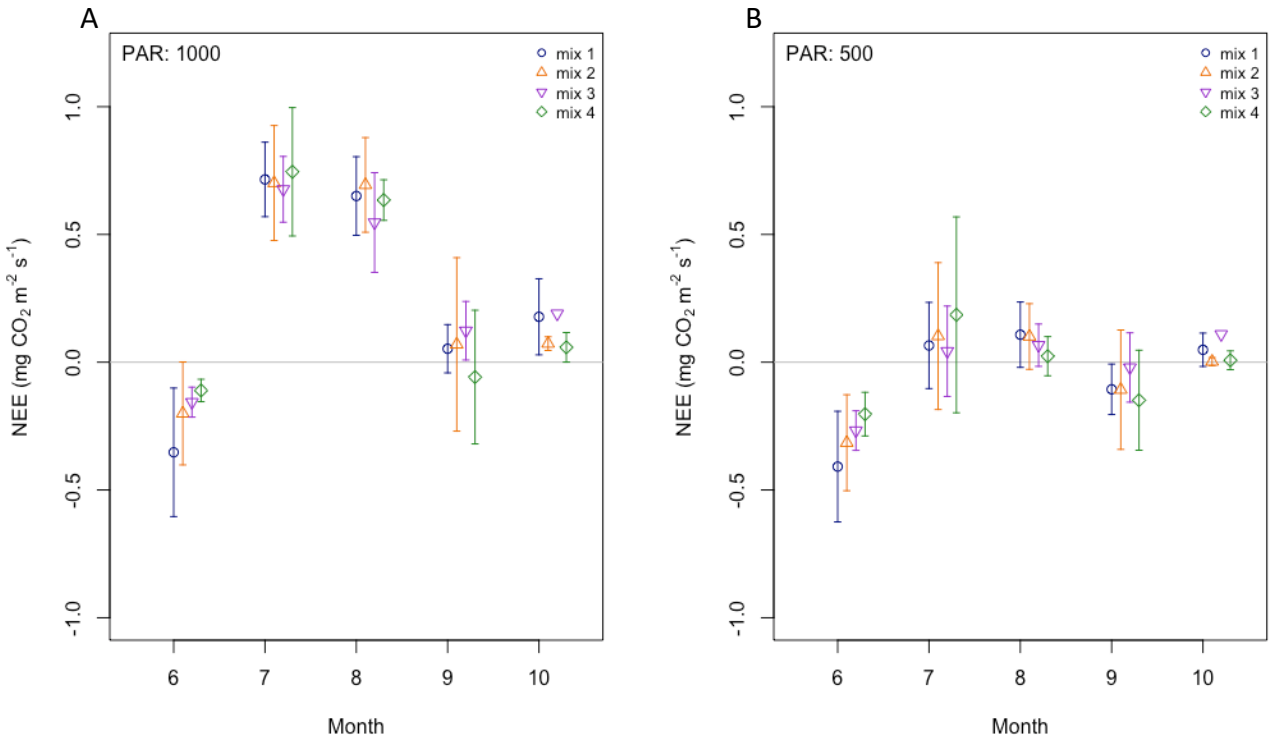

Figure 14. Net ecosystem exchange (NEE) when photosynthetically active radiation is A) 1000, and B) 500 µmol m^{-2} s^{-1}, mean ± standard deviation for all mixtures

3.4 Methane and nitrous oxide

Measured methane fluxes stayed relatively stable from June to September, being more often a sink than a source (Figure 15B). The treatments did not differ from each other since overall there was more variation within each treatment than between them. In September, there was a clear difference between mixtures 3 and 4. This was the case also in August but only 9 of 16 collars were measured. According to the statistical analysis, there were no significant differences between the treatments, with the results for September being closest to significant ($p > 0.1$).

All treatments acted as N_2O sources in June but the emissions levelled closer to zero by mid-July (Figure 15). According to the statistical analysis, the treatments did not significantly differ from each other. ANOVA for the number of species was not significant for any of the months but p-values were lower than with methane. The results were closest to significant in the first measurement of July ($p = 0.0519$) with a negative correlation between the number of species and the N_2O emissions.

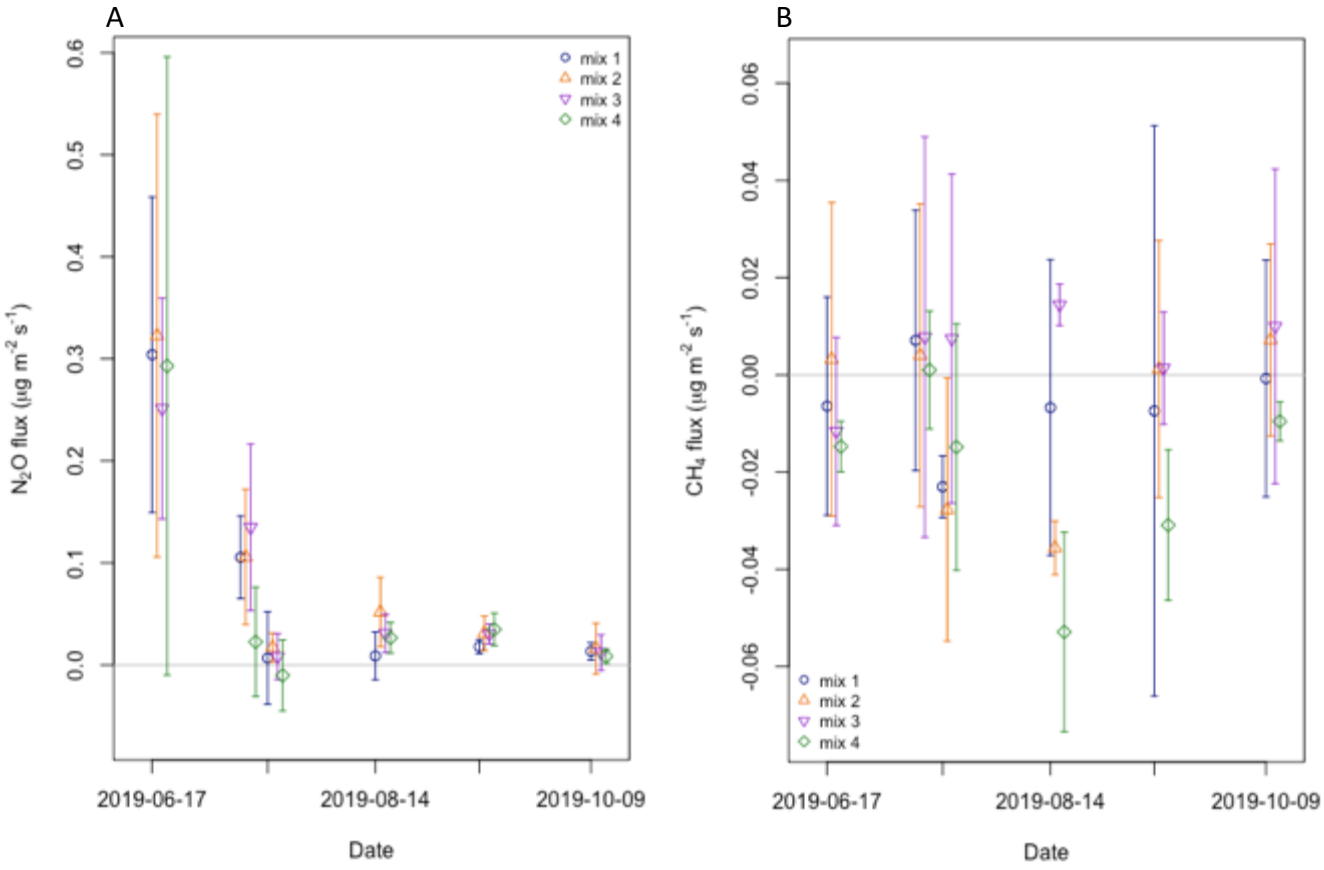

Figure 15. (A) Nitrous oxide and (B) methane flux mean ± standard deviation for all mixtures

4. Discussion

To find out the potential of species rich grass mixtures for mitigating climate impact of cultivation on organic soils, different grass mixtures were established with barley as a nurse crop and biomass, CO_2 exchange and the emissions of other greenhouse gases were monitored. In general, there were no statistically significant (p-value < 0.05) differences between the treatments in any of the measured variables, but there were some indications that, given more time after removing the nurse crop, some benefits might emerge.

4.1 Biomass and community composition

Overall, the species richness had no statistically significant effect on biomass due to the small scale of the experiment or the limited time the communities had to establish. However, there are some trends indicating that more species lead to higher productivity. In some cases, traits other than the diversity in the ecosystem have more influence into productivity (Hooper 1998). In this case the relatively short growth period after barley and possible variation in shading between the sampled points might be more important. Hence, according to this study, the biomass increased significantly with increasing diversity index, when treating the lowest biomass values as outliers that have suffered from overall poor growing conditions.

The complementary resource use concerning light, water, and nutrients (Hooper 1998) might be clearer when the biomass is closer to maximum so the exclusion of the lower values could be justified. Since all the space for growth is not filled to the full extent, the biomass is controlled by other factors. In these conditions, the growth is limited primarily by external factors, such as low radiation, low temperature, and allocation to cold hardening for winter. This affects all species mixtures equally and all of the biomass cannot grow enough for complementary effect being detectable.

One benefit of diversity is better adaptation to varying conditions (Solbrig 1994), thus some effects may be apparent only after several years. For example, Ranaldo et al. (2020) found that the benefits of diverse cover crop mixtures to ecosystem function were greater during a more unfavourable year. There was a long dry period during the measurements but not severe drought in the soil. This may have the barley, affected more so the differences in the rest of the biomass are not very clear. The correlation between species richness and biomass also been found to increase over time in perennial grassland with more plots performing better than the most productive monocultures (Tilman et al. 2001). Eisenhauer et al. (2012) suggested that changes in soil biota could explain the lag in response of productivity to diversity.

The evenness of the community seems important because the statistical model worked better with diversity index as explanatory variable. The Shannon diversity index is used because it shows the effect of both rare and abundant species (Morris et al. 2014), and some of the biomass samples of the ten-species mixtures were expected to have a relatively small amount of some of the species of

interest sown there. Morris et al. (2014) suggest using more than one index to have a better understanding of the different factors of diversity. This could be considered in future research.

The inclusion of belowground biomass would make the analysis more complete, but this is practical only after the barley roots have decomposed enough to separate them from the cover crop roots. Especially in the biomass of October there could be some samples skewing the distribution downwards (Figure 10C), but the sample is too small to be sure. At least the correlation seems to be clearer when only the largest amounts of biomass are considered.

4.2 Carbon exchange

The differences in carbon dioxide exchange between the different treatments were not statistically significant, probably because the differences in species richness measured in biomass were not significant either. Due to the small number of collars measured, a similar effect as with the small sample size in biomass results was in place. These results are contrary to a previous study, in which higher net carbon assimilation and allocation to shoots have been detected during establishing year (Stocker et al. 1999). Ecosystem respiration has been found to slightly increase in more diverse grass communities, but this is countered by clear increase in GPP, leading to more diverse system being net C sink (Milcu et al. 2014). Even though the results in this work were not significant, the trends are somewhat similar concerning respiration and photosynthesis.

The chamber measurements can be affected by the uncertainties in fitting the light response curve correctly. In addition, possible uncertainties in the respiration measurement will likely affect the light response curve, making the parameter values more uncertain. The sample areas inside the collars were different to the ones used for biomass, so uncertainties with measurement and effect of collars on microclimate may mask the differences more than in the biomass samples.

Since the use of diversity index made the differences between the biomasses more significant, similar consideration could be of use also with carbon. However, in this study a coverage analysis of the grass mixtures would have been difficult most of the time, because of small size of the grasses and the barley obscuring the view. Milcu et al. (2014) found that functional diversity affected the carbon fluxes mainly through more diverse leaf nitrogen concentrations in the canopy. Similarly,

Hirota et al. (2010) suggested that canopy structure could explain why species richness explained GPP better than respiration on an alpine meadow. The unfavourable measurement conditions for the chamber measurements, such as frosty mornings and cloudiness during the months of September and October, may obscure some of the effects expected after the removal of the barley. Especially differences in light capture would not be observable between relatively sparse communities in low light intensities of autumn.

Overall, the problem seems to be that after the barley was removed, the only measurements with enough viable chamber results for statistical analysis were in September. Apparently, one month was not enough time for the communities to develop after the removal of barley. Presumably, the unfilled space for growth had even larger effects on the community in September than in October as shown in the biomass results. An alternative explanation proposed to explain the higher productivity detected in more species rich communities is sampling effect, in which random selection of species leads to likelihood that more productive species are chosen when more species are sampled for (Huston 1997). In this experiment, this was countered by selecting two similar 5 species combinations and combining these to the highest 10 species mixture.

Surprisingly, the photosynthesis results of June seemed to have the most significant correlation to the number of species. It is possible that some species in the more diverse mixtures started the growth earlier and were more productive at the point they were measured but this effect would probably be quite small. Here the nearly significant results at the early stage could be interpreted as supporting the idea that a mixture with more species had the resources at the site more fully in use at early stage. This may be complementarity in timing of the use of resources or seedling responding differently to a neighbour of different species. Neighbours of different species have more varied effect on an individual than the neural or negative effect of neighbours of same species, making possibility of positive interactions more likely (Armas and Pugnaire 2011). For example, the facilitative interactions of legumes start already before litter could be significant, so root exudates also play a role (Armas and Pugnaire 2011).

4.3 Other greenhouse gases

Overall, there were no clear patterns in other green-house gas emission between the different treatments. The difference in methane emissions between mixtures 3 and 4 could indicate either that the species shared between mixtures 2 and 4 or diversity itself improves CH_4 sink or reduces CH_4 emissions. The main cause for methane emissions methanogen activity in anoxic condition, which is not directly dependent on species richness. In theory, a higher photosynthetic rate could lead to higher transpiration, but this is likely not measurable since water is free to flow to and from the experiment as well as between the treatments. Moreover, the soil temperature, which also have an effect on microbial activity, could be affected by a varying amount of shading, but for most of the high radiation period, the soil was shaded mainly by the nurse crop instead of the species mixtures.

However, methane emissions depend also on methanotroph activity which reduces the emissions. A grater methane sinks have been observed when plant diversity is increased from the level of conventional agriculture towards that of a natural forest (Levine et al. 2011). Additionally, in an Andean peatland, methane emissions were lower in sites with higher plant diversity (Villa et al. 2019). However, this study having only one measurement without the barley, is likely not sufficient to draw strong conclusions. In addition, the great variation in methane emissions in mixture 1 does not support these ideas.

Nitrous oxide emissions were explained better with species richness than methane emissions. However, the correlation was not consistently negative of positive. In general, N_2O emissions could be reduced when diversity is higher with more organic matter in the soil and better nutrient capture while increased emissions due to higher N turnover rate are also possible (Blesh 2019). In context of this study, there is already a lot of organic matter in the soil, so the effect of species richness is probably more mediated through N turnover and plant nutrient capture. If both are present, they could reverse each other, leading to no net effect to be detected, especially, since the measurements were relatively infrequent. This kind of interaction between factors that increase and reduce emissions could also explain the variation changing between negative and positive. N_2O emissions are very site specific (Piñeiro-Guerra et al. 2019) so to predict the emissions for other fields, the drivers of these difference need to be carefully considered.

One clear deficiency in this study was that the composition of species inside the collars was not estimated. Evenness of plant species may affect even CH_4 and N_2O emissions (Ribas et al. 2015) so a more careful study of emissions should consider the coverage of different species at the exact measurement spot. In addition, the time it takes for the methanotrophs to establish in restoration context can be quite long (Levine et al. 2011). Hence, if this kind of mechanism affected grass cultivation, it would not be in the time scale of this study. The same could hold true for N_2O to some extent.

4.4 Grass cultivation

The setup of this study could be considered analogous to cultivating cereal with a cover crop. However, choosing a good mixture of cover crop species may be different from a good mixture for a grass field, which is a predominant way of farming in the region where the study was conducted. Temporal variation in resource use was found to increase the yield more than different resource requirements in tropical intercropping (Fukai and Trenbath 1993). In this context, it may be more important in cereal cultivation to choose cover crops that can grow fast after the main crop is removed. On the other hand, when establishing grass field winter survival and long-term productivity are more important. Additionally, in long term in grass fields, the harvests act as disturbance. This can in itself help to maintain diversity as the dominance of the more competitive species is being limited (Grime 1973).

Presence of a few dominant species can be significant for the biomass production and up to 13 species can contribute detectably (Tilman et al. 2001). In the future, a study including more different species combinations, would allow analysing how the presence or absence of different species affects biomass and carbon sequestration. This would also allow considering the composition of species mixtures to secure the benefits of adding species. For example, the winter tolerance of different species is vital for successful cultivation in Northern latitudes. As an example, the seedlings of *Medicago sativa* tolerate cold better after dry soil conditions during hardening (Paquin and Mehuys 1980). A soil with high organic content may not be good for *M. sativa*, especially since it all but disappeared from the study site by summer 2020. In addition, testing different fertilization

levels and other possible benefits relating nutrient and leakage to aquatic systems might be useful to consider.

Productivity and CO_2 intake has been suggested to be better in an ecosystem with higher diversity on all trophic levels (Naeem et al. 1994). This has also been demonstrated in experimental setting (Weisser et al. 2015). For example, biodiversity in soil has been found to help in maintaining plant diversity (Yang et al. 2021), and genetic diversity within a species could be beneficial to species diversity and vice versa (Lankau and Strauss 2007). Since the grass fields are renewed every few years to keep the harvest levels up, the overall stability of ecosystem function could be significant. Even if the difference created were relatively small in a young grass filed, there is a possibility that more diverse grass community would be able to keep production up for longer. Reduced tillage and continuous vegetation cover have been suggested as an option for reducing emission where lots of the carbon is already lost and other mitigation strategies are not practical to implement (Kekkonen et al. 2019). Thus, renewing of a more species rich field could be less frequent, and this could reduce the emissions related to tillage.

4.5 Conclusions

Even though many studies have found evidence of diversity being beneficial to ecosystem function, in the scope of this study there was no strong evidence supporting species richness effect on biomass and greenhouse gas fluxes. However, the results indicate trends that can be useful for further studies. In the results concerning biomass the effect was clearer when the community composition was considered along the species richness and significant results emerged when considering a sufficient biomass for complementarity to have an effect. The differences in carbon flux measurements may be sensitive to timing within growing season and could benefit from including coverage analysis at least for functional types. Of the other greenhouse gases, species richness had most impact on early NO_2 emissions, while CH_4 flux probably needs significantly more time for any changes to appear. Overall, the effect of species richness needs to be studied in the long term to find out the effect over and possibly beyond the length of grass cultivation cycle. Based on current results species richness is unlikely to replace other methods of limiting emissions from cultivated organic lands but may be viable where these methods cannot be used for improving carbon sink of agricultural soils.

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Appendix 1. Names of the species

Table 2. Scientific, English and Finnish names for the species mentioned.

Scientific name	English name	Finnish name
Phleum pratense L.	Timothy	Timotei
Schedonorus arundinaceus (Schreb.) Dumort.	Tall fescue	Ruokonata
Festuca rubra L.	Red fescue	Punanata
Trifolium repens L.	White clover	Valkoapila
Medicago sativa L.	Alfalfa	Sinimailanen
Schedonorus pratensis (Huds.) P. Beauv.	Meadow fescue	Nurminata
Phalaroides arundinacea (L.) Rauschert	Reed canary grass	Ruokohelpi
Poa pratensis L.	Smooth meadow-grass	Niittynurmikka
Trifolium hybridum	Alsike clover	Alsikeapila
Cichorium intybus L.	Common chicory	Sikuri
Hordeum vulgare L.	Barley	Ohra
Elytrigia repens (L.) Desv. ex Nevski	Couch grass	Juolavehnä
Alopecurus geniculatus L.	Marsh foxtail	Polvipuntarpää

Appendix 2. Summary of statistics

Table 3. Statistics on biomass, gross primary production (GPP), respiration and net ecosystem exchange (NEE), nitrous oxide and methane fluxes. The more significant results are in black with colours getting lighter as the significance level drops.

			June		July		July 2		August		September		October	
			p	*correlation coefficient*	p	*correlation coefficient*	p	*correlation coefficient*	p	*correlation coefficient*	p	*correlation coefficient*	p	*correlation coefficient*
Biomass		log_e species richness							0.369	*41.8*			0.119	*16.1*
Biomass		Shannon index							0.870	*-32.4*			0.0676	*22.6*
Biomass	**> 60 g m^{-2}**												**0.0390**	***20.6***
GPP	PAR 1000	log_e species richness	0.0507	*0.0852*	0.503	*0.0421*			0.789	*0.0187*	0.212	*0.0866*		
	PAR 500	log_e species richness	0.161	*0.0589*	0.309	*0.0968*			0.667	*-0.0204*	0.884	*0.00516*		
Respiration		log_e species richness	0.279	*-0.0687*	0.630	*0.0268*			0.381	*0.0301*	0.580	*0.0269*		
NEE	PAR 1000	log_e species richness	0.0651	*0.154*	0.853	*0.0153*			0.864	*-0.0114*	0.814	*0.0260*		
	PAR 500	log_e species richness	0.0969	*0.128*	0.543	*0.0700*			0.265	*-0.0505*	0.765	*-0.0217*		
N2O		species richness	0.955	*-9.86 e-07*	0.0519	*-1.18 e-05*	0.354	*-2.50 e-06*			0.101	*1.92 e-06*	0.612	*-6.80 e-07*
CH4		species richness	0.529	*-1.20 e-06*	0.745	*-7.95e-07*	0.766	*7.24e-07*			0.24	*-3.47e-06*	0.451	*-1.48e-06*